# SALT AND WATER ANATOMY, CONSTANCY AND REGULATION IN RELATED CRABS FROM MARINE AND TERRESTRIAL HABITATS

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Among the numerous species of brachyuran crabs may be found some which are terrestrial, others which are semi-terrestrial and many which are marine. Within this definitive group of animals of close morphological and taxonomic similarities there is a spectrum of adaptation and the implied regulation of salt and water. This adaptation has succeeded across the marine-terrestrial path of emergence which has proved an insurmountable barrier to all but a few animals. Three species were selected to represent three different degrees of exposure of the animal to the electrolyte and water environment of the sea. The relationship between the electrolyte concentration of the marine environment and that within these animals was investigated to determine the degree of independence and the direction, degree and pathways of electrolyte and water regulation.

The common land crab, *Gecarcinus lateralis* (Frem.), was selected to represent the greatest independence from the marine habitat. It is found in burrows sufficiently high in the banks of beach sand on Nonesuch Island, Bermuda, that these burrows at their deepest do not approach within a meter of the high tide level. Nocturnal and beach scavenger in habit, it is able to go weeks, or even months, without entering the surf. The ghost crab, *Ocypode albicans* (Bosq), selected to represent a somewhat closer relationship to the marine habitat, is found in burrows near and above high tide level on the Delaware ocean beaches. These burrows approach and many have been found to penetrate high tide level with consequent flooding. A nocturnal beach scavenger, it goes into the surf briefly during feeding. The mangrove crab, *Goniopsis cruentatus* (Latr.), almost continuously in water, was selected to represent the closest relationship to the marine habitat. It is found in burrows in the silt and coral basins of mangrove swamps on St. George's Island, Bermuda. It leaves the burrows to seek food at night and may leave the water for brief periods by climbing out on mangrove roots. Seldom found more than a meter above the water and seldom more than two meters from a usable burrow, this sojourn into air appears superficially to be, timewise, a reciprocal of the air-surf relationship shown by ghost crabs.

Gross weight changes are the most obvious indicators where massive inboard or outboard water shifts are suspected, but in box-like animals such as the brachyuran crabs, unilateral water shifts and resulting weight changes may be

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expected to be of small magnitude. A second indicator might be blood specific gravity shifts resulting from the movement of water into or out of the circulating fluid as a result of electrolyte and osmotic imbalance, this indicated by a greater or smaller fraction of the blood being water.

The absence of gross changes in fresh weight or blood specific gravity does not preclude the possibility of electrolyte and water movement, but instead suggests that this movement results in constant water volumes and electrolyte concentrations. Although chloride ion concentration measurements in the environment, in the blood and in the urine give clear evidence of regulation of concentration in the ghost crab (Flemister and Flemister, 1951), the problem of rate and direction of exchange is difficult, if not impossible, to approach from the chloride ion concentration alone. Rate and direction of exchange may be determined by the use of an ion suitably alike to chloride in its distribution and biological properties in the range of concentrations required for analysis, yet subject to precise measurement apart from chloride. If used in sufficiently small quantities, the resulting environmental, blood and urine concentrations will not interfere with normal chloride movements which would be occurring at the same time, in the same direction, and, presumably, at about the same rate. It is assumed on the basis of an extensive mammalian literature that thiocyanate ions may be used to measure the space into which chloride ions are distributed, this space being profitably termed "extracellular," although some cellular absorption and concentration of both ions are known to occur in some animals. Such concentrations would involve only a limited portion of the data presented here, for this investigation is concerned primarily with the rates of exchange of ions and water between blood and environmental fluids. This is, in effect, a matter of using SCN as a "tagged chloride ion." A second indicator substance is necesary for such a study: a biologically inert non-electrolyte of minimal osmotic effect in the required concentration and of known pattern of movement across certain membranes relative to the movement of water. Inulin clearance is taken to indicate the rate of movement of fluid across the membrane of the antennal gland in a manner which will be called "filtration" for the sake of brevity. The simple filtration of inulin is assumed on the basis of osmotic and hydrostatic measurements on *Carcinus* by Picken (1936), inulin blood: urine ratios of unity found in the lobster, *Homarus*, by Forster and Zia-Walrath (1941) and by Burger (1955, 1957), and the possibility that the work of Maluf (1941), originally thought to indicate inulin secretion in the crayfish, *Cambarus*, may be interpreted differently as pointed out by Martin (1957).

The measurement of chloride, thiocyanate, inulin and water content of environment, blood and urine might be expected to illuminate the constancy of volumes and of electrolyte and water proportions, the direction and rate of movement of electrolytes and of water in the maintenance of the constancy against variation in the proportion of electrolyte to water in the environment.

## Methods

Ghost crabs (*Ocypode albicans*) from the Delaware beaches and land crabs (*Gecarcinus lateralis*) from Nonesuch Island, Bermuda, were kept in individual containers in which there was enough beach sand to allow burrow digging. Mangrove crabs (*Goniopsis cruentatus*) from mangrove swamps on St. George's

Island, Bermuda, were kept in individual containers with fresh sea water about two inches deep and planks on which they could get out of the water. Ghost crabs were fed all the fresh fish they would eat each night and allowed to swim in sea water for about five minutes. The once-a-day feeding and bathing routine paralleled natural conditions and made it possible to keep the animals in good condition for ten days or longer. At Bermuda, where fresh land crabs and mangrove crabs could be obtained more easily and at more frequent intervals, no effort was made to sustain a large number of crabs in the laboratory. Individuals of each species were in good condition after a week or ten days. All crabs were weighed daily and all had been in the laboratory at least twenty-four hours before any work was done with them.

The total water content of the animals was determined as the difference between fresh weight and the constant weight of the minced carcass after drying at 105° C. and cooling. Blood specific gravity of ghost crabs was determined by the method of Jacobsen and Linderstrom-Lang (1940) and blood total water was determined as the difference between fresh and dried weights of 1- to 2-cc. blood samples. None of the crabs used for these determinations were used in any other procedure.

Blood concentrations of thiocyanate and inulin, determined at 30-minute intervals on animals kept in dry containers during the three hours following injection of known amounts of the compounds, were used as the basis for extrapolation to the concentrations which would have been produced by complete and instantaneous distribution. The indicated concentrations were used to calculate the volumes available for SCN and inulin dilution. The variability of these volumes was appraised in relation to the total body water volume in 8 to 12 animals of each species. The blood and urine chloride concentrations and their variability were determined on a similar number of animals in dry containers. This quantitative characterization, the fluid and chloride anatomy, was used as the basis for the demonstration of regulation of electrolyte and water proportions in animals exposed to environmental salt and water stress. The rates of SCN and inulin loss and of SCN absorption were used to determine the rate, direction and pathway of electrolyte and water movement in these stress situations.

One-tenth of a cubic centimeter of blood was drawn from the sinus within the proximal joint of one of the legs, using a No. 27 needle fitted to a clean, dry one-quarter cubic centimeter tuberculin syringe in a holder. No anticoagulant was used. It was found that quick, smooth handling of the blood could effect the measurement and transfer of aliquots before clotting commenced. Aliquots of this blood sample were prepared as blanks for reference setting of the spectrophotometer for SCN and inulin measurements and the determination of control chloride concentrations. Injection of either 0.100 to 0.200 cc. 3% NaSCN (Merck Reagent), 0.100 to 0.200 cc. 5% inulin (Pfanstiehl C. P., re-crystallized), or 0.100 to 0.200 cc. 5% inulin in 3% NaSCN was made deep into the same sinus from a fixed-delivery syringe and needle. Immediately the same volume of the same solution was introduced into a 5-, a 10- and a 25-cc. volumetric flask, made to volume and samples taken from these were analyzed along with the blood samples for the precise determination of the amounts of NaSCN and inulin injected. Slow, deep introduction of injected fluid and careful sampling from deep within the sinus usually prevented fluid or blood loss from the site of puncture. In the few cases where fluid loss or bleeding did occur, the animals were discarded.

At fixed intervals after the injection of SCN and inulin, one-tenth of a cubic centimeter of blood was drawn from the sinus of the proximal joint of one of the legs on the side opposite the injection site. Two 0.500-cc. samples of diluted blood were prepared by transferring 0.040 cc. blood to 3-cc. test tubes, each containing 0.460 cc. distilled water. The transfers were made by separate, clean, dry measuring micropipettes of the Folin type which were flushed into the 0.460 cc. distilled water with repeated rinsing of the pipette lumen with the resulting 0.500 cc. of diluted blood. To one of the samples of diluted blood was added 1.00 cc. 10% $CCl_3COOH$, the mixture shaken thoroughly, centrifuged, and 1.00 cc. of the supernatant fluid transferred to a Coleman cuvette. Two-tenths of a cubic centimeter 10% $Fe(NO_3)_3 \cdot 9\ H_2O$ in 5% $HNO_3$ was added with thorough mixing, and the optical density of the resulting $Fe(SCN)_3$ was read immediately at 490 m$\mu$ and the SCN concentration calculated. This procedure is a modification of a method introduced by Crandall and Anderson (1934). On the second sample of diluted blood a Somogyi (1930) precipitation of protein was carried out by adding 0.50 cc. 10% $ZnSO_4 \cdot 7\ H_2O$ with thorough mixing and then adding 0.50 cc. 0.5 N NaOH, the mixture mechanically shaken for 30 minutes, centrifuged, and 1.0 cc. of the supernatant transferred to a 9-cc. test tube. Following in principle a method introduced by Young and Raisz (1952), 0.25 cc. 4 N NaOH was added with thorough mixing, the tube closed by a glass marble, and the contents heated in a boiling water bath for 15 minutes. The contents were cooled, and 6.25 cc. anthrone reagent, 0.4% anthrone (Matheson, Coleman and Bell) in 75% $H_2SO_4$, were added with cooling. The tube was again closed by a glass marble and the contents heated in a 75° C. water bath for 5 minutes, cooled, allowed to stand 30 minutes at room temperature, transferred to a Coleman cuvette, the optical density read at 630 m$\mu$ and the inulin concentration calculated.

From the sample remaining in the syringe and needle after the transfer of the two 0.040-cc. portions, blood was drawn to the 1 mark in a Thoma pipette and diluted to the 11 mark with distilled water. The contents were mixed and blown into a small glass cup with repeated rinsing of the pipette lumen. Duplicate 0.020- or 0.100-cc. aliquots of the diluted blood, depending on chloride concentration, were transferred by a micro blood sugar, Folin, pipette to 0.200 cc. distilled water in each of several depressions in a Coors porcelain plate with rinsing of the micropipette lumen with the now doubly diluted blood. Two-tenths of a cubic centimeter of 1 N $H_2SO_4$ was added. With mechanical stirring, 0.010 N $AgNO_3$ was added in small increments from a Scholander micrometer burette (Scholander *et al.*, 1943) and the potentiometric titration of the chloride ion was accomplished by the method of Cunningham, Kirk and Brooks (1941). Appropriate blanks and the determination of known standards accompanied the measurements of all unknowns.

Urine, collected as described by Flemister and Flemister (1951), was diluted in a Thoma pipette, aliquots taken and chloride, SCN and inulin concentrations determined by the procedures described for blood. Appropriate dilutions were made to hold concentrations within the sensitive range of the methods.

After determination of sea water chloride concentrations by the method described for blood, dilutions were made with distilled water and concentrations were accomplished by evaporation at room temperature to prepare environmental fluids containing 120, 240, 360, 480, 600 and 720 mM. Cl/L. To this series, which was

checked for chloride concentration after preparation, was added distilled water, presented in the graphs as 0 mM. Cl/L. Data from animals exposed to air rather than environmental fluids are presented as "dry." Animals were exposed individually to the various environmental fluids. They were placed in glass containers with sufficient volume, 200 to 300 cc., of the fluid to cover their bodies in the resting position. Fluids were renewed every twelve hours. Environmental solutions were prepared for SCN uptake by adding 1.00 or 2.00 cc. 3% NaSCN to each 100 cc. of environmental fluid. The amount added was fixed by the expected rate of absorption in order to keep blood concentrations within reasonable physiological limits and within the sensitive range of the procedures used for measurement.

## Results

Fresh weights of land crabs (*Gecarcinus lateralis*), 15 to 45 gm., ghost crabs (*Ocypode albicans*), 20 to 50 gm., and mangrove crabs (*Goniopsis cruentatus*), 20 to 50 gm., were random in distribution with no relation to sex or time of year. Gravid females were not collected. All animals were in the inter-molt period during the time they were in the laboratory. There was no significant weight gain or loss in ghost crabs maintained in the laboratory for as long as three weeks, nor in land crabs and mangrove crabs kept in the laboratory for a week or ten days. All variations in individual weights during captivity were less than 2.8% of the first weight determined soon after capture.

There was no appreciable, consistent change in fresh weight after 72 hours in any of the environmental fluids (120, 240, 360, 480, 600 and 720 mM. Cl/L.) except distilled water (0 mM. Cl/L.). In land crabs and ghost crabs exposed to distilled water for 24 hours and in mangrove crabs exposed for 48 hours, increases in weight never exceeded 4.8% of the fresh weights before the animals were placed in the environmental fluid. In view of the 2.8% variation in fresh weight of crabs in the control group and the difficulty of removing environmental fluid from the gill chambers before weighing in air, these weight changes are not considered significant.

No correlation was found between sex, size, or time of capture and blood specific gravity in 152 recently caught ghost crabs.After the initial determinations, 46 crabs were placed on sand and about 20 in each of the environmental fluids. Among the 46 crabs, after 72 hours on sand, the mean of specific gravity was 1.0442 with a standard error of 0.0009 and the mean for blood total water was 89.2% with a standard error of 0.29. Of about 20 crabs exposed to each of the environmental fluids, only in those surviving 24 hours in distilled water was there a possibly significant change in blood specific gravity, a decrease, with a "P" value between .01 and .05.

### *I. Water and electrolyte anatomy*

Reliable data on volumes of fluid and electrolyte concentrations within the animal are essential to an attempt to determine rates of exchange of water and electrolytes. Measurements of total water content and volume of fluid available for dilution of thiocyanate and inulin on 8 to 12 crabs of each of the three species studied are presented in Table I in terms of per cent of fresh weight. The second

TABLE I

*Water and electrolyte anatomy of land crab (Gecarcinus lateralis), ghost crab (Ocypode albicans) and mangrove crab (Goniopsis cruentatus)*

| | Land Crab | Ghost Crab | Mangrove Crab |
|---|---|---|---|
| Total water | | | |
| % fresh weight | 66.18 | 69.93 | 65.44 |
| S. E. | .52 | .70 | .63 |
| | 64.69–67.66 | 67.49–72.37 | 63.46–67.42 |
| SCN space | | | |
| % fresh weight | 28.52 | 34.18 | 30.39 |
| S. E. | .26 | .58 | .56 |
| | 27.79–29.25 | 32.18–36.18 | 28.60–32.18 |
| % total water | 43.1 | 48.9 | 46.4 |
| Inulin space | | | |
| % fresh weight | 18.80 | 21.49 | 19.92 |
| S. E. | .34 | .39 | .57 |
| | 17.84–19.76 | 20.13–22.85 | 18.67–21.17 |
| % total water | 28.4 | 30.7 | 30.4 |
| % SCN space | 65.9 | 62.9 | 65.5 |
| Blood chloride | | | |
| mM. Cl/L. | 385 | 378 | 422 |
| S. E. | 9 | 7 | 3 |
| | 360–410 | 354–402 | 413–431 |
| Urine chloride | | | |
| mM. Cl/L. | | 455 | 602 |
| S. E. | | 13 | 10 |
| | | 409–501 | 571–633 |
| Sea water | | | |
| mM. Cl/L. | 600 | 480 | 600 |

item under each tabulation is the standard error of the mean. The third item in each case is calculated from the standard deviation and is the range within which two-thirds of the data falls, this indicating the variability of the data making up each of the means. Applying the "t" test to the data and taking "P" values less than .01 to indicate significance, .01 to .05 possible significance, and values greater than .05 no significance, the following statements can be made. Although there is a significant, but not marked, difference between the SCN spaces, the volumes of the three compartments in terms of fresh body weight are much alike between land crabs and mangrove crabs. The volumes of the compartments of ghost crabs in the same terms are significantly, and markedly, larger with the exception of the inulin space as compared with that in mangrove crabs. Here the significance of the difference is questionable.

Compared as fractions of total water, the SCN and inulin spaces, presented as the fourth item in the tabulation in each case in Table I, show no significant differences in the three species studied. Therefore, absolute volumes, though showing the differences described in terms of per cent fresh body weight, are of comparable size relative to the total water content of the animal. The average of the means of SCN space is 46.2% and of inulin space 29.8% that of the total water content.

The average of the means of inulin space is 64.7% that of SCN space, fifth item of tabulation, with the fractions for land crabs and mangrove crabs being close together and somewhat larger than for ghost crabs.

Blood chloride concentrations of mangrove crabs are significantly greater than those of land crabs and ghost crabs, in which the concentrations are alike (Table I). A significant difference was found between urine chloride concentrations of ghost crabs and mangrove crabs immediately after capture. The greater concentration in mangrove crabs is to be related to their almost continuous exposure to sea water of high chloride content. Urine samples could not be obtained from land crabs. Within each of the three species, no correlation was found between sex, size, or time of capture and the fluid space available for dilution of SCN or inulin, the total water content, or the concentration of chloride ion in the blood or urine.

*II. Evidence of regulation*

The effects of exposure to distilled water (0 mM. Cl/L.), 120, 240, 360, 480, 600 and 720 mM. Cl/L. sea water dilutions and concentrations for 24, 48 and 72 hours on blood and urine chloride concentrations in the three species of crabs

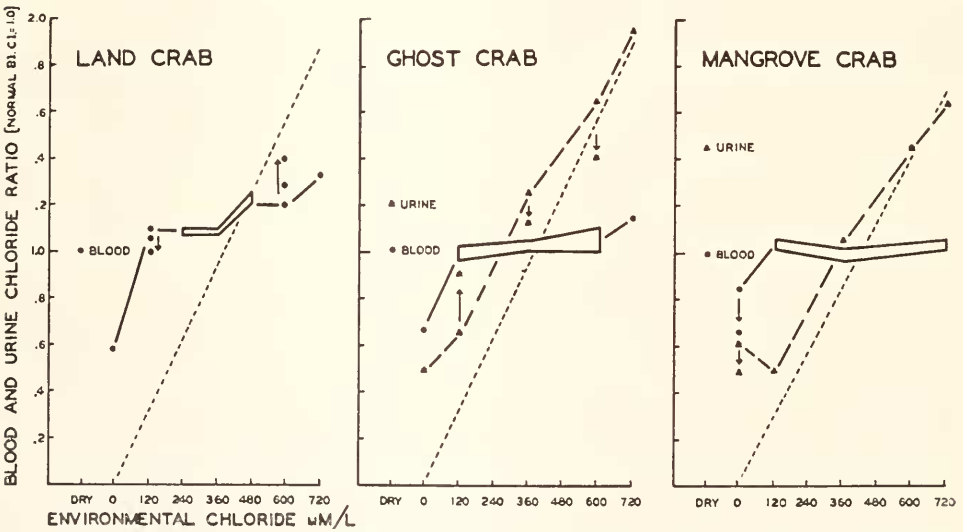


FIGURE 1. Blood and urine chloride concentrations of land crab (*Gecarcinus lateralis*), ghost crab (*Ocypode albicans*) and mangrove crab (*Goniopsis cruentatus*) related to environmental chloride concentrations.

studied are summarized in Figure 1. The normal blood chloride for each individual animal of each species on sand or in dry containers, plotted as "dry" and placed opposite "1.0" on the ordinate, serves as the basis for representation of the concentrations of chloride ion after exposure to experimental environmental fluids. The mean of the values for normal urine chloride and for blood and urine chloride ion concentration of the same 6 to 10 animals after exposure to experimental fluids, expressed separately as fractions or multiples of the normal blood chloride for the

same animal, is plotted for each experimental condition and time interval. Environmental fluid chloride concentrations are represented by a dotted line, blood chloride by a solid line and boxes are used to indicate ranges and concentrations of no statistically significant change during exposure, and urine chloride by a broken line. Additional points and arrows indicate positions and directions of shift of blood and urine chloride curves during exposure from 24 to 48 hours and from 48 to 72 hours. Standard deviations of these individual items of data were relatively small and are not indicated to avoid congestion of the graphs.

Data from land crabs present a picture of effective regulation of blood chloride concentration for 72 hours in mid-range environmental concentrations, though elevated by 10% in 240 and 360 and by 20% in 480 mM. Cl/L. fluids. A breakdown appears before 24 hours in distilled water, soon after 24 hours in 720 mM. Cl/L. with no survivors at 48 hours in either of these environments, and between 48 and 72 hours in 120 and 600 mM. Cl/L. fluids. Blood chloride regulation is somewhat more effective in hypotonic (except 0 mM. Cl/L.) than in hypertonic ranges, but in either, once the breakdown occurs, blood chloride levels approach environmental fluid concentrations. Urine samples could not be obtained from land crabs.

In ghost crabs blood chloride regulation is effective up to 72 hours over the range from 120 to 600 mM. Cl/L. environmental fluids, maintaining concentrations which do not differ significantly from those found in animals on sand. However, regulation fails during the first 24 hours in 0 and 720 mM. Cl/L. fluids. No ghost crab survived to 48 hours in these environmental extremes. Urine chloride concentrations roughly parallel, but are much higher than, environmental levels in all except the high concentrations (600 and 720 mM. Cl/L.). The antennal gland clearly wastes chloride in isotonic and hypotonic environmental situations. Urine chlorides vary little for 48 hours from 120 to 600 mM. Cl/L., but as exposure is prolonged to 72 hours, urine chloride concentrations approach blood chloride levels.

The blood chloride concentrations of mangrove crabs show a striking constancy at near normal levels over the range from 120 to 720 mM. Cl/L. environmental fluids for up to 72 hours. The slight elevations at the extremes fail statistical tests for significance. However, in animals exposed to distilled water, blood chlorides are significantly decreased in 24 hours and markedly so in 48 hours with no survivors at 72 hours. Urine chloride concentrations are very close to environmental levels in 600 and 720 mM. Cl/L. fluids, significantly above environmental levels in 360 and 120 mM. Cl/L. and are markedly elevated in distilled water. In crabs exposed to distilled water, urine chloride concentrations decreased between 24 and 48 hours, but still remained high. Urine chloride levels of animals in all other fluids did not change in 72 hours. These crabs fall short of the classical picture of completely effective regulation only in the breakdown in distilled water and the apparent leakage of chloride in the urine when exposed to 120 and 360 mM. Cl/L. environmental fluids.

### *III. Evidence of regulatory mechanisms*

Data on simultaneous inulin clearance and thiocyanate loss and on absorption of thiocyanate from the experimental environmental fluids into the blood of crabs of the three species studied are summarized in Figure 2. In the clearance and loss curves,

SCN and inulin concentrations of blood samples, taken at 1- to 4-hour intervals for the first 12 hours of exposure to environmental fluids (0 to 720 mM. Cl/L.) and then every 8 to 12 hours, are presented as fractions of the concentrations determined immediately prior to exposure and following a post-injection equilibration period of 2 to 4 hours in dry containers. Thiocyanate absorption curves are composed of blood SCN concentrations, determined at similar intervals and expressed as fractions or multiples of environmental SCN levels, in crabs exposed to environmental fluids (0 to 720 mM. Cl/L.) containing small amounts (4 to 7 mM./L.) of NaSCN, after a 2- to 4-hour period in dry containers. Corrections

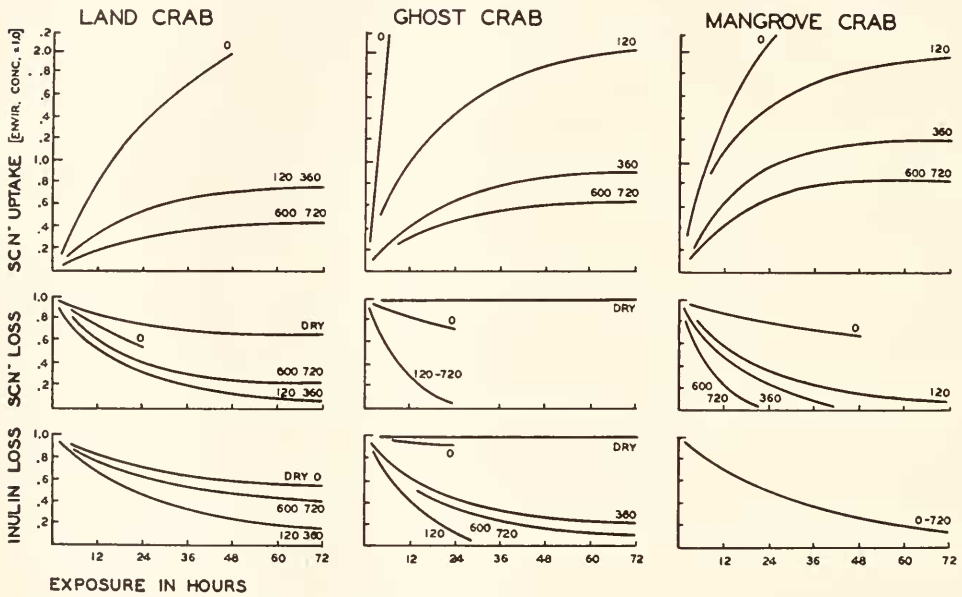


FIGURE 2. Inulin and thiocyanate loss and thiocyanate uptake by land crab (*Gecarcinus lateralis*), ghost crab (*Ocypode albicans*) and mangrove crab (*Goniopsis cruentatus*) exposed to a variety of environmental chloride concentrations.

were made in each case for SCN and inulin, or SCN alone, removed from the animal in taking samples. Standard deviations are not represented. They were relatively small except in very low and high concentrations. Sufficient numbers of animals, 4 to 18, were exposed to each of the experimental conditions to achieve statistically significant ("P" value of less than .01) separation by the "t" test. Determinations were made on fresh animals which were discarded at the completion of the 72-hour measurements.

In land crabs on sand, inulin clearance indicates continuing filtration with the rate of inulin loss slowly and steadily decreasing as blood level falls. Thiocyanate loss, comparable to inulin loss at first, decreases completely as blood concentration levels off, suggesting that SCN is re-absorbed from the fluid which continues to be filtered. The constancy of blood chloride concentration and of blood SCN levels after 24 hours indicates re-absorption of filtered water. In distilled water, inulin is cleared at the same rate as on sand, but SCN blood level falls more rapidly

and to about half the beginning value in 24 hours. This is comparable to chloride loss in distilled water and indicates a breakdown in electrolyte regulation not dependent on filtration through the antennal gland. Uninjected animals in 0 mM. Cl/L. fluid containing 4 to 7 mM. SCN/L. absorb SCN at such a rate that despite concurrent loss, which must be assumed, blood levels equal environmental concentrations in 15 hours and are twice as high in 48 hours. Though striking, this absorption results in accumulation of only about 7 mM. SCN/L. during the 24 hours when blood chloride levels fall 162 mM. Cl/L. Land crabs in 120 and 360 mM. Cl/L. fluids clear inulin more rapidly than those on sand or in distilled water, indicating a more rapid turnover, absorption and excretion, of water. In these fluids, SCN loss, more rapid and more complete than inulin clearance, suggests a pathway other than filtration. Blood SCN concentrations of uninjected crabs in these fluids, to which small amounts of SCN were added, are comparable and become steady at 80% of environmental SCN concentration in 48 hours. In all 600 and after 24 hours in 720 mM. Cl/L. fluids, inulin clearances and SCN losses are more rapid and more complete than those on sand or in distilled water, and less rapid and less complete than those in 120 or 360 mM. Cl/L. environments. Again, SCN loss rates, greater than inulin clearance rates, suggest a pathway other than filtration for electrolyte loss. Absorption of SCN from these environmental fluids, containing small amounts of NaSCN, are the same during the first 24 hours and at such a rate that blood SCN levels off at 45% of environmental concentration in 48 hours in animals exposed to 600 mM. Cl/L. fluids. The implication is clearly one of turnover rates reduced from those in hypotonic and near-isotonic fluids.

There appears to be a virtual shutting off of filtration in ghost crabs in the dry situation, judging from the fact that neither inulin, thiocyanate, nor chloride concentrations fall significantly during 72 hours on sand. It was increasingly difficult to get urine samples as exposure to a dry environment was prolonged. This suggests that water gained during brief nightly excursions into the surf is critical for adequate urine formation. The loss of SCN, far exceeding that of inulin in the 24 hours these crabs survived in distilled water, is parallel to the drop in blood chloride level, and is much greater than can be accounted for by a failure in re-absorption after filtration. Absorption of SCN added in small amount to 0 mM. Cl/L. fluids, reaching 3.7 times environmental concentration in 12 hours, 6.2 in 24 and 8.0 in 48, with no survivors at 72 hours, was even more striking than in land crabs, and indicated that 25 mM. SCN/L. was retained in the blood in the 24 hours while 129 mM. Cl/L. was being lost. In 120 mM. Cl/L. fluid, inulin and SCN disappear from the blood at about the same rate with complete removal in 30 hours. In this time sufficient SCN is absorbed from SCN-containing 120 mM. Cl/L. fluid to bring the blood level to 1.5 times the environmental level. This indicates a rapid water and electrolyte turnover with a somewhat excessive retention of ions from the environment which may be compensatory to the loss of chloride due to inadequate re-absorption by the antennal gland. Inulin clearance and SCN loss in 360 mM. Cl/L. environments indicate a much slower filtration and an electrolyte loss, complete in 30 hours, by a route other than filtration. Blood SCN levels in uninjected animals in SCN-containing 360 mM. Cl/L. fluids become constant in 48 hours at about 90% of environmental SCN concentration and at a time when urine chloride level drops from a high toward the environmental, and

blood, concentration. In all 600 and for 24 hours in 720 mM. Cl/L. environments, filtration rate is intermediate between those of animals in 120 and 360 mM. Cl/L. fluids and is inadequate to account for SCN loss which is complete in 30 hours. Blood SCN levels, resulting from absorption of SCN added in small amount to these fluids, are the same for the first 24 hours and reach a steady level in animals exposed to 600 mM. Cl/L. fluids at 60% of the environmental concentration in 48 hours.

Inulin clearance rates for mangrove crabs are the same for environmental fluids from distilled water to 720 mM. Cl/L. This indicates a remarkably versatile adjustment of chloride ion concentration in urine, if the regulation of the internal environment is to be maintained, as it apparently is, in contrast to widely different chloride concentrations in the environment. During 48 hours of exposure, SCN loss in distilled water is much less rapid than in the other environmental fluids, less rapid even than inulin clearance, indicating re-absorption of electrolyte after filtration. Thiocyanate absorption from SCN-containing 0 mM. Cl/L. fluid, much more rapid than that from more concentrated environmental fluids, results in blood SCN levels 2.2 times the environmental level in 24 hours, 2.8 in 48 and 3.0 in 72. The 9 mM. SCN retained in the blood at 24 hours does not compare favorably with the blood chloride loss, 72 mM./L., and the difference becomes greater by 48 hours. Thiocyanate loss rates, exceeding inulin clearances, become greater as environmental chloride concentrations increase from 120 to 600 and 720 mM./L., indicating electrolyte loss by a pathway other than filtration. Blood concentration of SCN absorbed from 120 to 600 and 720 mM. Cl/L. fluids containing small amounts of NaSCN has not reached a plateau after 72 hours in 120, has become steady at 1.2 times environmental level in 360, and at 80% of environmental concentration in 600 and 720 in 36 hours.

## Discussion

The absence of significant changes in the fresh weight of crabs exposed to environmental fluids of a wide range of electrolyte concentration makes it apparent that the volume of fluid within the animals is held constant although exchanges occur. The persistence of normal blood specific gravity in the ghost crab under such experimental conditions further indicates that there is no appreciable change in water or salt content of blood. Only after 24 hours in distilled water did this constancy of body weight and blood specific gravity show any signs of weakening. The significance of even these changes was questionable. The presence of regulation, therefore, is obvious.

Although there are statistically significant differences between the volumes of thiocyanate space, inulin space and total body water in the three species studied, there is no apparent correlation with dry or wet habitats. A lack of fundamental differences in the partitioning of water, SCN and inulin spaces and the implied cellular space, becomes apparent when these volumes are related to the volume of total body water.

The blood chloride concentration in the mangrove crab (*Goniopsis cruentatus*), significantly higher than those in the land crab (*Gecarcinus lateralis*) and the ghost crab (*Ocypode albicans*), bears a correlation to this crab's almost continuous exposure to sea water of high salinity. This correlation is also found by compar-

ing mangrove crab and ghost crab urine chloride levels. It is interesting that urine taken from ghost crabs soon after capture on Nonesuch Island, Bermuda, did not differ appreciably in chloride content from that taken from the ones captured on the Delaware beaches. The difference in salinity of the sea water available to the two habitats does not impose a difference in urine chloride clearance. This might be expected in view of the brief nightly exposure to the surf during feeding. However, mangrove crabs, constantly exposed to 600 mM. Cl/L. sea water, did show the effect of high environmental salinity.

During the first two hours after injection, inulin became diluted in a volume of fluid about two-thirds the indicated thiocyanate space. This suggests that either (1) the blood SCN after injection is less concentrated, indicating a larger dilution volume, due to absorption of SCN by cells, or (2) inulin more slowly penetrates the remote spaces invaded more rapidly by SCN. The similarity of the slope of the dilution curves for massive and light SCN injections and the similarity between simultaneous SCN and inulin curves suggest that only the mechanical factors of spreading are involved. Recovery determinations on ghost crabs, accounting for 87 to 97% of injected SCN under a variety of environmental conditions, indicate that little, if any, SCN is bound by cells. Whether or not inulin eventually invades all of the SCN volume can only be suggested on the basis of data presented here. The apparent cessation of antennal gland activity in ghost crabs on sand appears to offer some opportunity for an answer. So far, it appears that in the 70 hours following the first two, inulin still occupies only two-thirds of the SCN space. The suggestion of a functionally closed circulation, inulin space, within the larger extracellular compartment, SCN space, is an interesting one for which the mechanical factors of lumen flow and stream boundary diffusion seem reasonable.

The breadth of the range within which two-thirds of the data are estimated to fall, presented in Table I, is taken to be a reliable indication of the effectiveness of regulation. Comparison of these ranges reveals that the three fluid compartments, total water, SCN space and inulin space, are more closely regulated in land crabs than in ghost crabs and mangrove crabs. A greater difference in the regulation of these volumes might be expected between ghost crabs and mangrove crabs in view of the difference in the stress imposed by their normal habitats. Chloride concentrations in the blood and urine of mangrove crabs are much more closely regulated than in land crabs and ghost crabs. This indicates that the land crab is farther along in the evolution of volume regulation and that the mangrove crab has a more definitive control of chloride concentration.

Comparison of chloride and SCN loss from the blood of crabs exposed to the various environmental fluids shows that these ions move at about the same rate in each species and in each situation. Urine SCN concentrations stood in the same ratio and range to blood SCN levels as did these respective concentrations of chloride. The graphs summarizing data are not further complicated by adding these items, inasmuch as they duplicate the chloride data. These observations indicate that it is valid to use SCN as "tagged chloride" in an effort to determine the movement of chloride ions under conditions of electrolyte and water stress. The presence of inulin or SCN in the blood did not affect the clearance, rate or degree, of the other. Inulin was not absorbed from the environmental fluids. Its presence in the environment did not affect the rate or degree of absorption of

SCN from the environmental fluids, or the rate or degree of inulin or SCN clearance from injected animals. This was true even when sufficient inulin was added to the environmental fluids to make them equal in concentration to the blood of animals injected for the determination of inulin clearance. Therefore, inulin was judged to exert no appreciable effect on the direction, rate or degree of electrolyte and water shifts in the concentrations used. The presence of SCN in the environment in concentrations used did not affect the rate or degree of inulin clearance, but it did affect the rate of fall of SCN levels in injected animals. In injected animals placed in fluids to which SCN had been added, blood concentrations of SCN fell more slowly and only to a point well above equilibrium with environmental SCN in 120 mM. Cl/L., about equal in 360 and well below in 600, but not cleared.

Of the three species, only the land crab and the ghost crab survived 24 hours out of water. This was to be expected from the differences in habitat and was one basis on which the three species were selected. The inulin and SCN clearance in land crabs and ghost crabs on sand for 72 hours, during which blood chlorides remained constant, indicate a difference in antennal gland activity. The indicated re-absorption of filtered water in land crabs could account for the lack of obtainable urine. If the re-absorption of electrolytes is obligatory, it could be a cause of the elevated chloride levels found in land crabs exposed to hypertonic environments. In ghost crabs, such a continuing filtration and re-absorption does not appear to exist. The dependence on contact with the sea for filtration and resulting urine formation is in agreement with the observations by Burger (1957) that haemoconcentration, from keeping lobsters in air, suppresses urine formation. His interpretation is that non-diffusible molecules in the blood draw in water principally through the gills, and that this water is bailed out as urine.

The similarity between inulin clearance rates in land crabs on sand and for 24 hours in distilled water is interesting. The same is true of ghost crabs. The persistent high inulin concentrations in these latter animals suggest very little filtration in distilled water. The possibility is immediately obvious that cellular osmotic swelling in gill membranes and branchial epithelium may cause mechanical, if not metabolic, interference with absorption of water by crabs in such environments. If this should be the case, why are mangrove crabs different?

Chloride and SCN loss in 0 mM. Cl/L. fluid, most rapid in land crabs and least so in mangrove crabs, appears to be compensated for by the absorption of available ion, SCN, from the environmental fluids. The rate and degree of net gain, blood concentration, of the absorbed ion is not proportional to the rate or degree of blood chloride, or SCN, loss. The three species clearly differ in their ability to retain normally present chloride ions and to absorb and hold SCN ions. The blood chloride level in land crabs seems to be least well held and the least well protected by absorption rates. The blood chloride of ghost crabs is somewhat better held and is better protected by a remarkably rapid absorption rate. Retention of blood chloride in mangrove crabs, best of the three, is supported by an intermediate absorption rate. The apparent superiority of the holding and compensatory mechanisms in mangrove crabs is reflected by their longer survival, past 48 hours. It should be pointed out, however, that in all three of these species, the net absorptions are inadequate to compensate for a falling blood chloride. The significance of some ion, however dilute, to the survival of crabs in 0 mM. Cl/L.

fluids is shown by the doubling of survival time by the retention in the blood of 7 mM. SCN/L. for 162 mM. Cl/L. lost in land crabs, 25 for 129 in ghost crabs and 9 for 72 in mangrove crabs during the first 24 hours of exposure. There was no such increase in the survival time of crabs in 720 mM. Cl/L. fluids to which similar amounts of Na SCN had been added. None of the animals showed any signs of depression.

Urine chloride of ghost crabs exposed to distilled water for 24 hours was 48% of blood concentration, indicating that some, though obviously not all, filtered chloride is re-absorbed. This is also indicated by comparable SCN data. The high urine chloride is not high enough to suggest chloride secretion by the antennal gland. The impression that these animals formed very little urine is supported by the fact that only 10% of the injected inulin is cleared during this 24-hour period. The less-than-blood concentration and the small volume of urine and the loss of one-third of blood chloride suggest a removal of chloride, and SCN, from the blood by a pathway other than the antennal gland. The urine chloride concentration in mangrove crabs, 62% of blood level, after a similar exposure indicates partial chloride recovery by the antennal gland. This re-absorption continues through 48 hours, but fails to repair a falling blood chloride concentration.

Comparison between a 35-gram ghost crab and a similar mangrove crab, calculated from data presented in Table I, serves to demonstrate the possible difference in pathways of chloride loss in animals exposed to distilled water. From Figures 1 and 2, it may be seen that 88 mgm. NaCl were lost from the blood of such a ghost crab during 24 hours in distilled water and that the concurrently formed urine contained 11 mgm. NaCl per cubic centimeter. For the chloride lost from the blood to have been cleared by only the antennal gland, 8.0 cc. of urine would have had to be formed. As calculated by inulin clearance, only 0.8 cc. of fluid was filtered during this period. Urine inulin concentrations were roughly equal to blood levels, indicating little or no water re-absorption or secretion after filtration. Ninety per cent of the chloride loss must have been by another route in the ghost crab. From Figures 1 and 2 it appears that 45 mgm. NaCl were lost from a comparable mangrove crab during a similar exposure and that the urine formed contained 15 mgm. NaCl per cubic centimeter. The filtration and excretion of 3.0 cc. of this urine would account for the blood chloride loss. According to inulin clearance, 3.5 cc. fluid were filtered, and according to urine inulin concentrations there was no appreciable re-absorption of water. In spite of the clearance of proportionately less chloride than water by the antennal gland, suggesting re-absorption of chloride, this is the only pathway necessary to account for the observed failure in chloride ion regulation in mangrove crabs exposed to distilled water. Re-absorption of chloride occurred in both species in distilled water. Since 0.8 cc. blood was filtered in the ghost crab, 18.0 mgm. NaCl crossed over into the lumen of the antennal gland. Since urine contained 48% blood chloride concentration, 8.6 mgm. NaCl were lost, and the remaining 9.4 mgm. must have been re-absorbed. The 3.5 cc. blood filtered in the mangrove crab carried 86.4 mgm. NaCl into the antennal gland. The urine, containing 62% blood chloride concentration, removed 53.6 mgm. NaCl, leaving 32.8 mgm. to be re-absorbed. This is in agreement with the observed chloride loss. The removal of more water than electrolyte from the blood of ghost crabs and mangrove crabs,

and the decrease in blood chloride concentrations of all three species exposed to distilled water for 24 hours, make it apparent that the water entering the animals is flushing chloride out through the antennal gland. Moreover, loss of chloride through another pathway is indicated in land crabs and ghost crabs, but not necessarily in mangrove crabs.

Although complete extraction of electrolytes in one passage through the gill chamber can not be assumed, comparisons of absorption rates can be made. The absorption of SCN added to 0 mM. Cl/L. environmental fluids indicates a withdrawal from a volume of environmental fluid equal to the SCN space, about 11 cc. for a 35-gram animal, in 2 hours for ghost crabs, 6 hours for mangrove crabs and 15 hours for land crabs. Leveling off of the concentration curves in time suggests that if absorption rates hold, the rate of diffusion outward increases with increasing concentration. This suggests a far more rapid turnover at the gill membrane and, perhaps, branchial epithelium than clearance rates in the antennal gland would indicate. The gill and, perhaps, branchial epithelium appear to be the site of this absorption activity since animals whose digestive tracts were closed at both ends with grafting wax did not differ in absorption rates from those animals not blocked. Similar blocking prior to electrolyte and inulin loss determinations indicated that the digestive tract has no significant role in the clearances observed.

The appreciable, 10% rise in blood chloride concentrations in land crabs exposed to 120, 240, and 360 mM. Cl/L. fluids indicates that the rate of absorption of chloride from these environments exceeds the rate of loss until a new steady-state is reached. The steadily maintained higher blood level, failing only in 120 mM. Cl/L. at 72 hours, shows that the regulation is effective, if not compensating. The much more elevated, 20% higher, yet steady concentrations found in animals exposed to 480 mM. Cl/L. for 72 hours, 600 for 48 and 720 for 24, indicate that this regulation persists and has some flexibility and upper limits in situations hypertonic to the blood. Absorption rates are greater than indicated by the concentration curves, for it must be assumed that during absorption the ions are being lost at rates suggested by the SCN loss curves. The slower rate of SCN loss from injected animals, the slower rate of SCN absorption by uninjected ones and the slower rate of filtration in 600 and 720 mM. Cl/L. fluids indicate that there is reduced exchange with the environmental fluids perhaps due to reduced exposure which in turn may be due to a partial restriction of gill chamber volume or flow as suggested by the work of Gross (1957) on the brachyuran shore crab (*Pachygrapsus crassipes*) exposed to hypertonic fluids. The loss of ions across the gill membrane and, possibly, the branchial epithelium and the persisting, though reduced, filtration through the antennal gland are not sufficiently rapid to prevent an accumulation of ions from the environment resulting in the elevated blood chloride level observed. Although the lack of urine data precludes further analysis and appraisal of this regulation, it appears that there is a correlation between the dry habitat of land crabs and their relatively slow electrolyte clearance resulting in elevated blood chloride levels even in hypotonic environmental fluids.

The regulation of blood chloride concentration in ghost crabs is more rigid from 120 to 600 mM. Cl/L. than in land crabs. The similarity of the SCN loss curves for 360 to 720 mM. Cl/L. fluids, faster than inulin clearance, indicates that the antennal gland is of only secondary importance in electrolyte loss in near-isotonic and hypertonic environments. Since in all fluids the injected SCN is cleared in

about 24 hours, the constancy of the blood chloride concentration would appear to depend on the net absorption, or retention of the same quantity of electrolyte irrespective of environmental concentration. This can be concluded to happen from the net absorption curves. Proportional to chloride ions present in environmental fluids, there is about three times as much SCN in 120 mM. Cl/L. fluids as in 360 and almost twice as much in 360 as in 600. This is approximately the ratio of net absorption concentration of SCN accumulating in the blood during exposure to the various environments. The greater volume of environmental water involved in this extraction process in 120 mM. Cl/L. fluids is reflected in the more rapid filtration through the antennal gland.

Blood chloride is held constant over a wider range, 120 to 720 mM. Cl/L., for 72 hours in mangrove crabs than in either of the other two species. The close approximation of urine chloride concentrations to those of environmental fluids suggests that the regulation is closely held and yet flexible in that absorbed ions are apparently retained in hypotonic situations and cleared in hypertonic ones. The fact that filtration continues at the same rate for all environmental fluids, even distilled water, shows that constant blood chloride levels must be maintained by prompt re-absorption of ions and water by the antennal gland and by absorption and loss by any other route of exchange involved. The more rapid loss of SCN in hypertonic environments than in near-isotonic ones, and these more rapid than in hypotonic ones, at a time when blood chlorides are constant, shows that the turnover, absorption and loss, of electrolytes is more rapid in the more concentrated environments. This may account for the fact that the accumulated SCN curves fail to level off at points which suggest the ratios of proportionate SCN and chloride concentrations, as was found in ghost crabs. The outbound passage of the same amount of water through the antennal gland in all environmental concentrations, indicated by inulin clearance, fails to account for electrolyte clearance, except from crabs in distilled water.

The fact that urine chloride concentrations approach environmental fluid levels and not blood levels during exposure to 120 to 720 mM. Cl/L. environments for up to 48 hours in ghost crabs and 72 hours in mangrove crabs suggests that the antennal gland re-absorbs some chloride in hypotonic and some water in hypertonic situations after filtration. It is apparent that the reabsorption of chloride is not completely adequate in hypotonic environments in either species and begins to fail earlier in ghost crabs than in mangrove crabs. The re-absorption of water in hypertonic environments is more effective in both species. Urine electrolyte and inulin concentrations indicate that the high urine chloride in hypertonic and near-isotonic environments is due to re-absorption of water. Inasmuch as blood chloride levels continue to be maintained, and inulin data indicate only a moderate increase in filtration and only a moderate decrease in water re-absorption by the antennal gland, the markedly reduced level of urine chloride in hypertonic environments at 72 hours implies a closing of a portal of entry of chloride in the ghost crab. This might be due in part to restricted gill chamber exposure suggested in the shore crab in hypertonic fluids by Gross (1957). The marked increase in urine chloride concentration in the ghost crab in 120 mM. Cl/L. fluid in 72 hours, when blood chloride level remains constant and urine inulin concentrations indicate no re-absorption of water, suggests accelerated chloride absorption from the environment. It is interesting that urine chloride concentrations in the two species

are near normal when the mangrove crab is in 600 mM. Cl/L. fluid, its normal habitat, and when the ghost crab is in 360 mM. Cl/L. fluid, near isotonicity with its blood. It is also interesting that urine and blood chloride concentrations are equal when the animals are exposed to environmental chlorides 100 mM. Cl/L. less concentrated than the blood. This gives a rough estimate of the re-absorption gradient in the antennal gland and indicates that similar mechanisms and thresholds are involved in the two species.

When animals of these three species are exposed to environmental fluids ranging from 120 to 600 mM. Cl/L., the rate of turnover, absorption and loss, of electrolytes and the rate of filtration are less in the land crabs than in the others. The difficulty in getting urine samples suggests re-absorption of most of the filtered water, which might be expected in view of this crab's adaptation to a dry habitat. The electrolyte turnover and filtration rates are most rapid in the ghost crab in hypotonic and in the mangrove crab in hypertonic environmental fluids. There is an apparent correlation between the almost constant exposure of the mangrove crab to sea water hypertonic to its own blood and a rapid turnover and clearance rate. It appears that the defense in the ghost crab is against the inbound movement of hypotonic fluids and that this is a poor defense at best in view of the inefficient re-absorption of chloride by its antennal gland. It is interesting that when animals of these species are exposed to environmental fluids which are near isotonic to their own blood concentrations, the filtration rates through the antennal glands are similar. This indicates that the hydrostatic and osmotic factors in filtration are similar in all three of the species. This augments the interpretation based on the uniformity of re-absorption gradients that similar mechanisms and thresholds are involved in antennal gland function in the three species.

In the early intervals of SCN absorption and loss determinations, before blood levels are much altered, absorption rates exceed loss rates in ghost crabs and mangrove crabs and are about equal in land crabs in hypotonic, 120 mM. Cl/L., fluids. Land crabs and ghost crabs hold about equal in near-isotonic, 360 mM. Cl/L., fluids, but mangrove crabs show an absorption advantage in the same fluid, which is hypotonic to their blood. Early loss rates exceed early absorption rates in all three species in hypertonic, 600 mM. Cl/L., sea water. These absorption and loss rate differences are parallel to the leveling-off points in the SCN accumulation curves, which are interpreted to arise from the equating of outbound and inbound passage of ions across gills and, perhaps, branchial epithelium as blood concentrations are increased as a result of absorption exceeding loss earlier. Comparison of these leveling-off concentrations of net absorbed, accumulated, SCN and the ratio of the concentration of chloride maintained in the blood and that imposed by the environmental fluid shows close agreement for near-isotonic and for hypertonic situations. In both the ghost crab and the mangrove crab, the plateau has not been reached in hypotonic fluid, but in the land crab there is evidence of both a leveling-off and a breakdown in blood chloride regulation at 72 hours. In crabs of all three species exposed to distilled water, the plateau is so remote and the breakdown so severe that no conclusions can be drawn. Leveling-off of loss curves in time is interpreted to reflect rates markedly reduced by the falling blood concentration. After ten days no SCN or inulin could be found in injected animals kept in the laboratory under normal conditions. The evidence from the net absorption curves is that electrolyte movement is rapid and precise. In hypotonic

environments an appreciably longer time is required to reach a plateau than is required to clear the ion once it is injected, which suggests that there is a choke on the rate of absorption of ions from hypotonic fluids. This is altogether reasonable when the handling of the required amount of fluid is considered.

The loss of injected SCN to a level well above a concentration in equilibrium with SCN added to 120 mM. Cl/L. environmental fluid coincides with and supports the evidence from urine chlorides of animals in such environments that electrolyte loss continues even in situations where ions must be acquired to maintain constancy. The fact that injected SCN falls to approximate equilibrium with environmental SCN in animals exposed to near-isotonic fluids also supports this evidence. The loss of injected SCN to a concentration less than environmental in animals in 600 mM. Cl/L. fluids, in which absorbed SCN is held to less than equilibrium concentration, shows that the capacity to lose electrolyte is not saturated by this degree of hypertonicity. It is clear that these loss rates are much greater than can be accounted for by passage through the antennal gland.

On the basis of early clearance rates, before blood concentrations are greatly decreased, fluid equal to the inulin space volume is filtered by the antennal gland of land crabs in their normal habitat on sand in about 60 hours, and in mangrove crabs in 600 mM. Cl/L. sea water, their normal habitat, in 24 hours. In ghost crabs on sand no appreciable filtration was found. However, in sea water between 360 and 600 mM. Cl/L., to which ghost crabs normally have access, the filtration rates are similar to those of mangrove crabs. This indicates the importance of the ghost crab's brief nightly exposure to the surf. It is assumed that in their normal habitat, ghost crabs filter somewhat slower than do mangrove crabs, but that they do filter is apparent from the fact that fresh-caught crabs have urine. Therefore, an obvious correlation exists between filtration rate and type of habitat in these three species.

The turnover rates indicate the activity in electrolyte and water movement which goes on during the maintenance of constancy of volumes and concentrations in the water compartments measured. The persistence of normal values for these quantities in the variety of devised and imposed environmental stress situations is as remarkable as the rate of continuous change which underlies it. It must be concluded that in submerged crabs of these three species, the gills and, possibly, the branchial epithelium provide the principal pathway for this rapid and precise absorption and loss of electrolytes and water, and that the antennal gland plays only a limited role in this turnover. However, the urine chloride, thiocyanate and inulin concentrations indicate that clearance through the antennal gland may provide the all-important fine adjustment in blood concentration of electrolytes and water.

Among these three species, found in different degrees of exposure to seas of different salinity, the mangrove crab, most constantly and continuously exposed intimately to the stable environment of the sea, is the one showing the greatest capacity to regulate the concentration of blood chloride when subjected to environments of widely differing salinities. The crab most independent of the sea, the land crab, has the most definitively regulated volume of total, inulin space and SCN space water, and an adequate electrolyte regulation when exposed to a limited hypotonic range or to food containing proportionately more water than salt, but little or none when environmental chloride exceeds that of blood as does the sea water accessible in its habitat. In the ghost crab, intermediate between them,

there appear to be the mechanisms for effective regulation with diminished chloride absorption in hypertonic fluids and increased absorption in hypotonic ones, but with the threat of an extravagantly wasteful chloride loss through the antennal gland. The independence of the land crab from the sea depends on the maintenance of a gradient and not on effective regulation. The land habitat of the ghost crab is critically dependent on access to the surf, albeit for a short nightly exposure. The sea habitat of the mangrove crab is a complete commitment despite a wide range of effective regulation. There emerges a picture of independence which depends on the constancy of a normal gradient, and the capacity to tolerate a changing gradient which depends on effective regulation. They afford the mechanisms of adaptation to totally different habitats.

## Summary

1. Exposure to environmental salinities ranging from 120 to 720 mM. Cl/L. for 72 hours did not produce changes in fresh weights of the land crab (*Gecarcinus lateralis*), the ghost crab (*Ocypode albicans*) or the mangrove crab (*Goniopsis cruentatus*). There was an increase in weight of questionable significance after 24 hours in crabs exposed to distilled water. Only in distilled water was there any change in the blood specific gravity of ghost crabs. Even this change was of questionable significance.

2. The total body water content of ghost crabs is significantly larger than those of land crabs and mangrove crabs, which are similar. The fractions of total water content which are available for the dilution of thiocyanate and inulin are similar in the three species. The volumes available for the dilution of inulin are about two-third the volumes in which SCN appears to be diluted. This suggests the interesting possibility of a functionally closed, lumen flow, circulation.

3. The blood chloride concentration of mangrove crabs, although less than that of their environment, is significantly greater than those of the more terrestrial ghost crabs and land crabs, which are similar. The urine chloride concentration of mangrove crabs is identical to that of its environment and is more concentrated than that of ghost crabs.

4. Exposed to environmental fluids of 120 to 600 mM. Cl/L. sea water for 72 hours, land crabs show adequate regulation of blood chloride concentration over a limited hypotonic range, but little or no regulation in fluids hypertonic to its blood chloride. Blood chloride regulation in ghost crabs is adequate over this range, but with the production of a urine which wastes chloride in hypotonic fluids. Mangrove crabs show an adequate and closely held regulation of blood chloride concentration in this range and the production of a urine with chloride levels similar to those of the environment, but with some chloride leakage in hypotonic fluids. Blood chloride regulation failed in all three species when exposed to distilled water for 24 hours, and in land crabs and ghost crabs exposed to 720 mM. Cl/L. for about 24 hours. Mangrove crabs survived 72 hours in 720 mM. Cl/L. fluid with regulation intact, but could not survive 24 hours in air.

5. On dry sand, land crabs filter across the antennal gland a volume equal to their inulin space in 60 hours. It also re-absorbs most of the water of the urine thus formed. This is not true of ghost crabs in which the formation of urine appears to depend on water gained during brief nightly exposures to the surf. When

exposed to 600 mM. Cl/L. sea water, their normal habitat, mangrove crabs filter their inulin volume in 24 hours. There is an apparent correlation between these filtration rates and the availability of water in the habitat.

6. Antennal gland filtration and re-absorption rates are adequate to account for the rate of chloride loss in mangrove crabs in distilled water. This is not true for ghost crabs and land crabs in which filtration rates are not much faster than those on sand. Electrolytes are escaping across some other membrane, supposedly gills and, perhaps, branchial epithelium. The loss of electrolyte by a route other than the antennal gland is also apparent in animals of all three species exposed to environmental fluids from 120 to 720 mM. Cl/L.

7. Re-absorption of chloride by the antennal gland of ghost crabs and mangrove crabs exposed to hypotonic fluids and of water in animals exposed to hypertonic fluids is apparent from the similarity between urine and environmental chloride concentrations. Similar re-absorptions can be inferred from data presented on land crabs.

8. The similarity of the mechanisms and thresholds involved in antennal gland function is indicated by (1) the approach of urine chloride concentrations to the blood chloride levels when ghost crabs and mangrove crabs are exposed to environmental fluid chloride levels 100 mM. Cl/L. less concentrated than the blood, and (2) the similarity in filtration rates in all three species when animals are exposed to environmental fluids which are near isotonic to their own blood chloride concentrations.

9. The blood concentrations of SCN absorbed from 120 to 720 mM. Cl/L. environmental fluids tend to plateau, due to equating of inbound and outbound ion passage, at a point roughly equal to the ratio between blood chloride and environmental chloride levels. The point of plateau is reached more slowly in hypotonic situations indicating the difficulty of handling the required volume of environmental fluid. The persistence of electrolyte loss, even in situations where ions must be rapidly absorbed to maintain constancy, is indicated by the SCN loss rate curves for the various environments.

10. The rates of turnover of water and electrolyte are as remarkable as the constancy of the regulation from which they result and for which they are responsible. The effectiveness of this regulation in mangrove crabs and the maintenance of a concentration gradient in land crabs can be related to the successful adaptation of these two species to totally different habitats.

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